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New South Wales
Field Ornithologists Club
Lecture

Tuesday, March 2: *How do YOU keep your records?* — Introduced by Walter Boles.

At Hallstrom Theatre,
Australian Museum at 8.00 p.m.

Founding patterns of *Exoneura bicolor* Smith in Cobboboonee State Forest, southwestern Victoria

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ABSTRACT

The study investigated the effects of pyrrhic succession on sociality and some life-history parameters of a facultatively social Australian native bee, *Exoneura bicolor*. In a comparison between trap nests and established colonies, it was found that established colonies contained, on average, more females and more brood per colony. There was no difference in the number of females per trap nest between areas at different stages of pyrrhic succession, although there was some difference in the proportion of trap nests colonized. The greatest proportion of trap nests colonized were in sites where fire had passed a year prior to the trap nests been set. Electrophoretic assay of the females in co-founded nests showed that relatedness between co-founders is not significantly different from zero, although the sample size was small. It was established that emus *Dromaius novaehollandiae* were responsible for breaking the inaccessible inflorescences from the nesting substrate, thereby allowing the bees access to the nesting substrate. These data support the hypothesis that *E. bicolor* are able to take advantage of the abundance of nesting sites that follow fire.

INTRODUCTION

Fire is an important feature of the Australian ecosystem, as is evidenced by the widespread adaptations of many Australian native plants to fire. To date there has been considerable research on the effects of burning on flora and vertebrate fauna, but comparatively little on terrestrial invertebrates. What has been done is mainly on fossorial invertebrates, both on leaf litter and the soil itself (Campbell and Tanton 1981; Suckling and Macfarlane 1983). Although invertebrates are sometimes inconspicuous, they form the largest proportion of faunal biomass and the effect of invertebrate herbivores and plant parasites on a habitat can be greater than that of vertebrate herbivores. Because of the importance of invertebrates in an ecosystem, it is necessary to understand the impact of fire on life-history and population parameters of these animals.

This study concentrates on the effects of fire on a plant pollinator, the native bee *Exoneura bicolor*, in Cobboboonee State Forest near Portland, Victoria. *E. bicolor* Smith is interesting because it is a plant pollinator in a fire-prone environment, and so must have experienced periodic fire for a long time. Additional data were also collected on the population densities of two other *Exoneura* species, *E. bicincta* Rayment and *E. (Brevineura) rufitarsis* Rayment.

E. bicolor is a common bee which nests in pithy stems throughout southeastern Australia and is found from montane open forest to heathland. Its nests consist of unlined, unbranched burrows that are not divided into cells (Schwarz 1986). Immatures are placed in the nest lumen, not confined to cells, and are provisioned progressively throughout their development by females within the colony (Schwarz 1987). In the Portland region the species nests in a number of substrates including the dried flower scapes of *Xanthorrhoea minor* and *X. australis* and the dead branches of *Melaleuca squarrosa* and *Eucalyptus* spp. For this study I examined only those colonies in *X. minor.*

Head (1987, 1988) estimated that Aborigines have occupied the Discovery Bay/Portland region for at least the last 7 000–8 000 years and used fire throughout that period. She states that although it is difficult to separate anthropogenic and natural influences on the vegetation, it is highly probable that Aborigines were using fire to influence vegetation and game movements to some extent. She found that the area was subjected to periodic fire (frequency unknown), which created a mosaic of different growth stages within the forest.

Silberbauer (1989) found that the social structure of *E. bicolor* colonies in the Portland region depended on the area in which they were found. In some areas the majority of colonies were multifemale and semisocial, while in other areas the colonies were predominantly solitary, although there seemed to be no difference between the habitats of the two colony types other than those due to variation in successional regrowth since the last fire (pyrrhic succession). Further investigation of these observations could help explain the importance of variation in environmental conditions in determining the social structure adopted by facultatively social insects. This has become an interesting problem in sociobiology as researchers question the contribution of environment in the evolution and maintenance of group living and sociality in Hymenoptera (Strassmann 1989). *E. bicolor* is facultatively social i.e., solitary, quasisocial and semisocial colonies may be found (Schwarz 1987). Examination of the differences between areas in which solitary and social colonies predominate may help to elucidate the effects of environmental variation on social diversity.

This study aims to investigate the effects of periodic fire on *E. bicolor* by measuring life history parameters, social variation and population dynamics in several microgeographic regions at differing stages of pyrrhic succession.

MATERIALS AND METHODS

Most of the data for this study were obtained by placing trap nests in the study sites for three months, after which the trap nests were collected and returned to La Trobe University to be opened and their contents examined. Trapped colonies rather than naturally occurring colonies were used because it is much easier to standardize the number of available nest sites (not the density of bees) using imported trap nests already existing at those study sites. This method does not take into account variation in the density of the bee population. Furthermore, there may be variation in nesting substrate quality between sites, and use of trap nests will remove this source of variation.

(1) *Study Area*

All the research was performed in Cobboboonee State Forest and Lower Glenelg National Park between October 1990 and April 1991. Cobboboonee State Forest consists of dry sclerophyll open forest interspersed with swamps and low heathland. The forest has been periodically logged and subjected to both natural wildfire and regular, prescribed fuel-reduction burns. Fire has created a forest with mixed-age understoreys at different stages of pyrrhic succession. This study utilized two study sites (1 and 2, Fig. 1) within

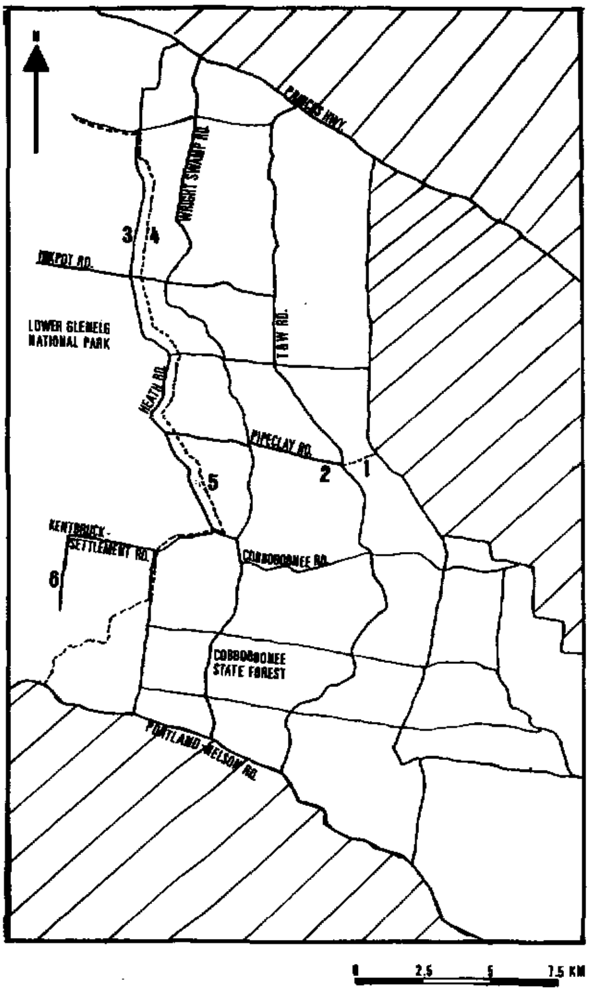

Figure 1. Map of Lower Glenelg National Park and Cobboboonee State Forest showing the location of study sites (numbered 1–6). – – – Border between Lower Glenelg National Park and Cobboboonee State Forest.

Cobbboboonee, both of which are in open forest and had been burnt in autumn 1989 and autumn 1990 respectively.

The eastern border of Lower Glenelg Park is partially continuous with Cobbboboonee State Forest. The major difference between the state forest and the national park is the absence of logging and fuel reduction burns in the latter. I used four sites within Lower Glenelg National Park (Fig. 1). Sites 3 and 4 were in open forest that was burnt in spring 1989 and autumn 1990 respectively and site 5 was a heathy meadow that was burnt in the summer of 1986. Site 6 was in Kentbruck Heath, a large heathland that had not been burnt for at least 15 years.

In the Cobboboonee region *E. bicolor* has been recorded nesting in a number of substrates. The study concentrated on those bees found nesting in *Xanthorrhoea minor* as the colonies are easy to find, plentiful, and *X. minor* scapes make effective trap nests. *X. minor* is common throughout the study area as both a component of the understorey and part of the heathland. Following fire it is one of the first plants to reappear, flowering *en masse* in the spring following the fire.

(2) *Trap Nests*

Previous work has shown that *E. bicolor* will readily establish colonies in trap nests made from the broken scapes of *X. minor* (Silberbauer 1989). Unoccupied *X. minor* scapes were obtained from nearby Mt Clay State Forest, broken to expose the pithy centre, and marked with coloured insulation tape. Trap nests were placed in experimental plots by inserting them into the bases of *X. minor* tussocks. Schwarz (unpubl.) found no differences in colonization rates between clumped or dispersed trap nests, so nests were clumped within *X. minor* tussocks to make recovery easier. Between one and three trap nests were placed in each tussock, depending on the size of the tussock. When *X. minor* flowers there may be as many as a dozen scapes per tussock (pers. obs.), so that placing more than one trap nest per tussock approximates the natural situation.

(3) *Established Colonies*

Approximately 86 naturally-occurring colonies in *X. minor* scapes were collected on March 13–14, 1991, from site 1 that had not been burnt for at least six years. Many of these colonies appeared to be more than one year old and it is likely that more than one generation of bees had lived in the nests. Whether or not broods have been raised in the nest can be estimated by examining the nest lining for traces of decayed pollen and nectar (Schwarz 1987). During brood rearing pollen balls are deposited by foragers for both the brood and the adult occupants of the nest and leave a yellowish mark on the nest wall that persists for many months, gradually becoming darker with age. The length of the nest may also be an indicator of the age of a colony; established nests usually have longer nest lumens (the hole excavated by the bees) than newly founded ones (Schwarz 1986). Only colonies that showed signs of being more than one year old were used for analyses.

(4) *Brood Experiment*

Three 10 × 10 m quadrats, each with 50 trap nests, were marked out in each of six study sites (Fig. 1): *site 1* (open forest, 1 year post fire), *site 2* (open forest, 6 months post fire), *site 3* (open forest, 1 year post fire), *site 4* (open forest, 6 months post fire), *site 5* (low heath, 4 years post fire) and *site 6* (low heath, 15+ years post fire). Trap nests were placed in late spring, November 13, 1990. At this stage the bees had just begun to fly and colonize new nests. The trap nests were left in the field until the end of the brood rearing period. They were collected on February 26–27, 1991, and opened at La Trobe University. At the end of summer (February/March) the brood are pupating and emerging (eclosing) as adults, but have not yet begun to leave the nest to found new colonies. This is also the only time that it is possible to readily distinguish newly emerged brood (callows) from older adults as newly emerged brood are lighter in colour, although the cuticle quickly hardens and darkens. For each site and quadrat I recorded the following: presence or absence of a bee colony, the species of any occupants, number of adult females, adult males, the number and size of any immatures and callows.

(5) *Electrophoresis*

In site 1, 13 trap nests contained two female co-founders each. In order to estimate within-colony relatedness the females were assayed electrophoretically at two presumptive loci. Electrophoresis was performed using starch gels and Poulik's discontinuous buffer system (Poulik 1957). Running and staining procedures for the two loci, staining as esterase (Est) and superoxide dismutase (SOD), are given in Blows and Schwarz (1991). Relatedness (*sense* Grafen 1986) was estimated using Queller and Goodnight's "Relate" programme (Queller and Goodnight 1989) and standard errors were obtained by jacknifing over colonies.

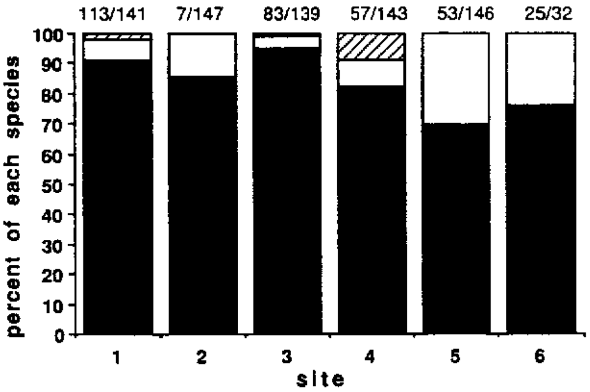

Figure 2. Graph showing the species composition in trap nests at each site as a percentage of the total number of occupied trap nests. Solid black represents *E. bicolor*, white represents *E. (B.) rufitarsis* and hatched represents *E. bicincta*. Above each bar is a ratio of the total number of occupied trap nests over the number of trap nests recovered at each site.

RESULTS

(1) *Species Composition in Trap Nests*

The number of each bee species found in the trap nests are presented in Figure 2. *E. bicolor* was the most common species. The other species present were *E. bicincta* and *E. (Brevineura) rufitarsis*. The occurrence of these latter species was patchy and the number of trap nests occupied was small (Fig. 2). One major problem encountered was retrieving the trap nests at the conclusion of the experiment. In one site (site 6) the heath was so thick that finding the comparatively small *X. minor* scapes was difficult. As a result, a small fraction of the 150 trap nests at this site were recovered. Recovery rates for all of the sites are given in Figure 2 as a ratio of the number of occupied nests over the number of nests recovered.

(2) *Comparison of the Number of Females in Trap Nests versus Established Colonies*

The numbers of adult females per colony in both established colonies and trap nests are shown in Figure 3a. For these analyses, trap nests from all sites were pooled. There were approximately three times as many trap nests collected as established colonies. There are significantly more females per colony in established colonies (mean ± S.D., 2.80 ± 1.57, n = 86) than newly founded colonies (1.10 ± 0.39, n = 291) (Fig. 3a).

(3) *Comarison of the Number of Brood in Trap Nests versus Established Colonies*

There were differences in the number of brood in established colonies and trap nests (Fig. 3b and 3c). It appears that there are more surviving brood in established, multifemale colonies compared with newly founded, single-female nests. The observed ratio of female to male pupae + callows is also different in the two types of colony. There is a greater proportion of male pupae in established than in newly founded colonies (6 per cent male brood in new colonies and 14 per cent male brood in established colonies, Fig. 3b). It is impossible to estimate the sex ratio of the entire brood as it is only possible to sex pupae and prepupae. At this stage it is not feasible to raise larvae to the pupal stage, when they can be sexed, so none of the larvae found at the time the nests were collected could be used to calculate sex ratio. There were adult males in many of the multifemale colonies, but it could not be assumed that all of these were newly eclosed brood from that nest, as it is known that males in a closely related species, *E. asimillima* Rayment, leave the natal colony quite soon after eclosion and start visiting neighbouring colonies in search of virgin females (Sugden and Pyke 1991).

(4) *Between-site Comparison of Number of Females in Trap Nests*

The numbers of females per trap nest in each site was compared. A Kruskal-Wallis test of the number of females per colony showed that there was one female per trap nest, regardless of site ($p > 0.05$, n = 347). In 90 per cent of the occupied nests there was one nest founder, regardless of the length of time since fire. This indicates that, unlike the montane population (Schwarz 1986), colonies are initiated by a solitary female.

(5) *Between-site Comparison of the Proportion of Trap Nests Colonized*

Although there were no significant differences in the number of females per colony, there was variation between sites in the proportion of trap nests that had been colonized. For these analyses site 6 was excluded because recovery rates were so low. A within-site, between-quadrat chi-square test was performed for each of the five remaining sites using the number of occupied trap nests. The results of the chi-square tests (Table 1) show that there were no significant within-site, between quadrat differences for sites 1, 2 and 3, but that quadrats in sites 4 and 5 were significantly different from each other ($p < 0.05$, Table 1). A comparison between sites, using the rate of trap nest occupancy, shows that sites where fire had passed a year prior to the study (sites 1 and 3) had the greatest proportion of nests colonized (Table 1). There was no similarity of colonization rate between sites where the fire had passed most

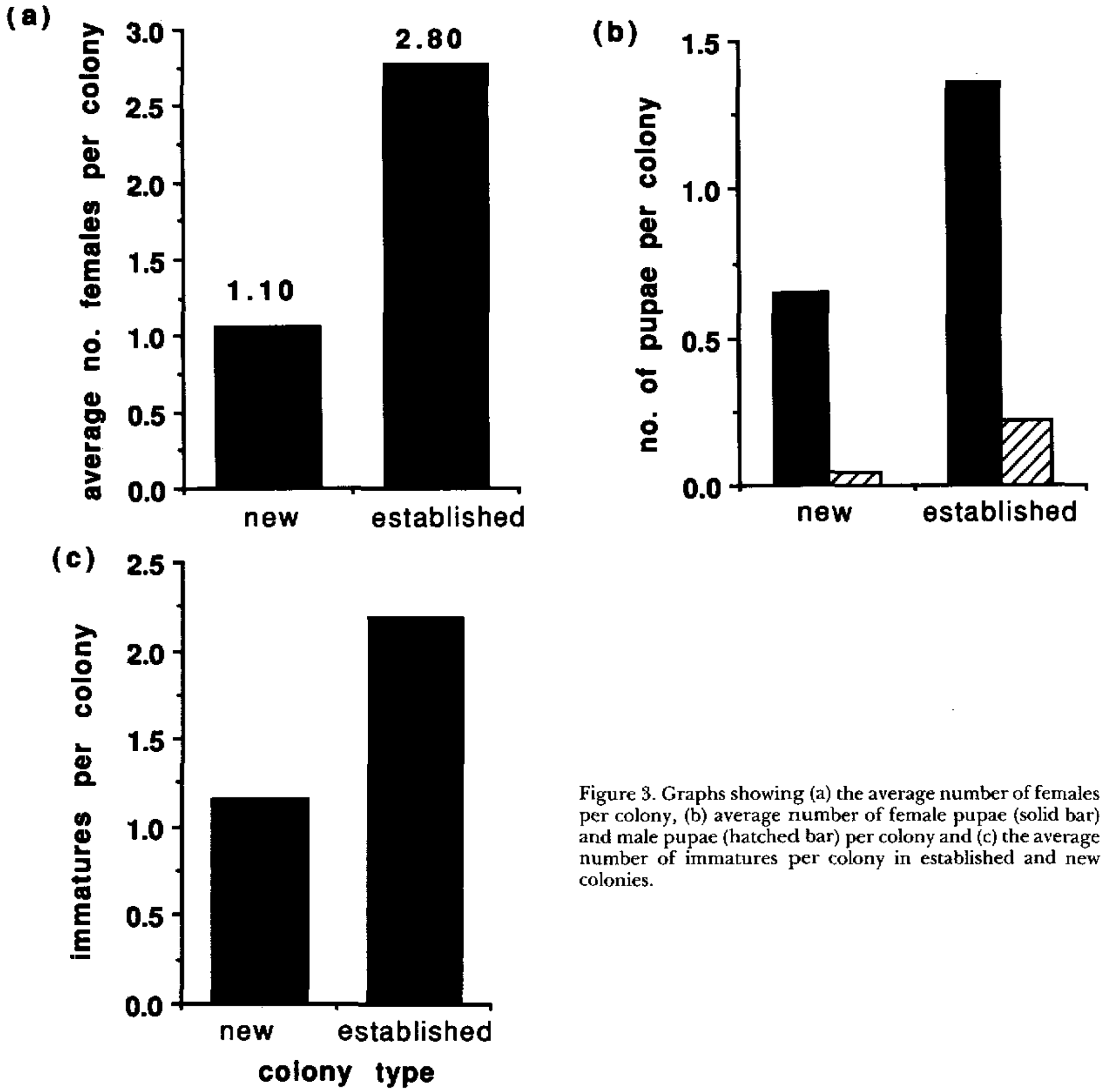

Figure 3. Graphs showing (a) the average number of females per colony, (b) average number of female pupae (solid bar) and male pupae (hatched bar) per colony and (c) the average number of immatures per colony in established and new colonies.

recently (sites 2 and 4, Table 1). The site where fire had passed through four years previously (site 5) had a rate of trap nest occupancy intermediate between the sites where fire had passed six months and a year previously (Table 1).

(6) *Starch Gel Electrophoresis of 2-female Colonies from Site 1*

Using the "Relate" programme (Queller and Goodnight 1989), the results of the starch gel electrophoresis of 13 two-female colonies (26 females in all) from site 1 show that relatedness is not significantly different from zero (−0.004 ± 0.322; r ± S.D., df = 12), although the standard deviation is high. The high standard deviation is probably related to the small number of loci and the small number of colonies. In contrast with the relatedness estimate, the inbreeding coefficient is high (0.32 ± 0.19, F ± S.E.). Larger samples are necessary before any firm conclusions can be reached.

(7) *Emu Observations*

It is not possible for *E. bicolor* to establish colonies in *X. minor* unless the pithy centre of the scape is exposed because they are incapable of breaking through the tough outer covering of the flower scape. The majority of naturally-occurring nest sites that had lost the top part of the scape seemed to have been broken off evenly, rather than the kind of breakage that agents such as wind would cause. A scape broken by an emu *Dromaius novaehollandiae* looks as though it has been shorn horizontally by secateurs. Consultation with local farmers indicated that emus may have been responsible

Table 1. The chi-square values for a within-site comparison of the number of colonized nests per quadrat for each site. Site 6 was not included in these analyses because recovery rates were too low to compare meaningfully with the other sites. Tot/site refers to the summed number of occupied nests per site, i.e., the rate of trap nest occupancy.

Sites	Time since burn	Tot/site	χ^2-value	P
Site 1	1 year	113	0.224	NS
Site 2	6 months	7	1.160	NS
Site 3	1 year	83	1.417	NS
Site 4	6 months	57	6.63	$p < 0.05$
Site 5	4 years	53	12.216	$p < 0.001$

for removing the inflorescences from the tops of the scapes. In order to verify this, observations using binoculars were made of feeding emus and their droppings were collected for analysis. Emus were observed feeding on the soft green *X. minor* scapes, and examination of the droppings collected showed that green *X. minor* inflorescences, along with grasses and bracken fiddleheads *(Pteridium* sp.) had been eaten by emus.

DISCUSSION

Results presented here show that there were significantly more females in established colonies than in newly founded colonies, and that the majority of new nests are excavated by a solitary female. Examination of established colonies showed that many were multifemale colonies. As colonies are mainly initiated by single females, the most likely method by which colonies grow is by offspring remaining in their natal colony.

The difference in the number of brood and males in established versus newly established colonies may be related to a number of factors. Obviously, there are more females in multifemale colonies and they may be able to produce more brood in total than solitary females. Other factors may also contribute to differences in the number of brood and number of males. There were more males present in established colonies than newly founded nests, and although adult males cannot be assumed to be brood from that colony, the difference between the two classes of nest may be indicative of local resource enhancement, similar to the montane population (Schwarz 1988). It is possible that established colonies produce more male brood than new colonies. It is also possible that the larger number of females in established nests act as more of an attractant to males than single-female colonies.

The most likely explanation of the larger number of brood in re-used, multifemale colonies than new, single-female colonies is the presence of extra egg-layers in multifemale colonies. It is also possible that the greater number of brood in re-used colonies is related to nest structure, i.e., that a re-used nest is longer (M. Schwarz, pers. comm.), therefore the brood at the bottom of the nest lumen receive greater protection from the weather, predation and parasitism. Established colonies are able to begin producing brood before dispersers are able to begin nest founding activity (Silberbauer 1989), so the brood-rearing season for new colonies is not as long as that for re-used nests. Another factor is the time and energy expended in excavation of new nests results in reduced potential brood production. Multifemale colonies enable task specialization, such as guarding and foraging, which may increase brood survival.

There was no significant difference found between the number of females in trap nests at different sites. Another between-site variable, however, was the proportion of the original 150 trap nests that were eventually colonized. The within-site variation in colonization rate observed may represent the natural patchiness of bee populations. It was expected that recently burnt areas would not only provide a large number of potential nest sites, but the relative vegetative simplicity and uniformity of these sites would facilitate high rates of colonization. Apparently, the vegetative complexity of unburnt sites compared with recently burnt sites does not explain observed between-site variation. At Cobboboonee there was no link between the length of time since fire and the rate of colonization.

A comparison between sites using the summed values for the quadrats within the sites showed that the greatest proportion of colonized nests were in the sites that had been burnt a year previously, but there were significant differences between sites that were burnt less than a year before the study. The site that had been burnt four years previously had lower occupation rates than sites that had been burnt a year prior to the study. This suggests that the number of colonizers in an area can be related to the time since disturbance. There is no remnant population of *E. bicolor* after fire because the fire destroys all nest sites in its path and no further nest sites are created for at least six months after the fire.

X. minor flowers in the spring following fire and emus eat the green inflorescences, exposing the pithy centre of the scape and creating nest sites for bees. Bees will not utilize the broken scapes until about a year after the tussocks have flowered, or at least until the scapes have dried enough to make excavation of a nest possible. The bees do not excavate nests in the relatively moist green stems (pers. obs.).

It is likely that the density of bees in a recently burnt area increases until checked by some factor, such as the shortage of nesting sites. Anecdotal observations (pers. obs.) suggest that the first multifemale colonies begin to appear in appreciable density after the area has been colonized for a year and females are starting to re-use nests. It is possible that this is because dispersing females are unable to find unoccupied nesting sites. Schwarz (unpubl. data) has found that the expected success of single-female colonies of *E. bicolor* in heathland is higher than in montane areas. In the areas where *X. minor* is the main source of nest sites, the number of colonies would begin to drop after 5–8 years, when the *X. minor* scapes start to deteriorate and become unsuitable as nest sites (pers. obs.). In a forest such as Cobboboonee where there are control burn fires almost every year, there are always patches in the forest where *X. minor* has recently flowered. There are also other nesting substrates in which *E. bicolor* can nest, like *Melaleuca squarrosa* and *Eucalyptus* spp., which do not appear to deteriorate as quickly, but do not provide as many nesting sites as *X. minor* because of the way nest sites are created in these substrates (pers. obs.).

Deliberate burning of areas to promote flowering of *Xanthorrhoea* species and to concentrate game by attracting it to new growth (J. Boura, pers. comm.) was probably performed over approximately 7 000 years (Low 1988; B. Gott, pers. comm.). It is possible that within this period the behaviour and colonization patterns of *E. bicolor* have been modified to take advantage of the periodical abundance of nest sites that follows fire.

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